EXCRETION OF THE METABOLITES OF TESTOSTERONE-4-C\textsuperscript{14} IN THE RAT\textsuperscript{*}

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In most of the experiments previously reported on the metabolism of testosterone, reviewed by Dorfman (1), rather large quantities of this steroid have been administered to experimental subjects and the urine collected for the estimation of androgenic compounds. In general, metabolites amounting to about 20 per cent of the administered compound have been found in the urine of animals. However, in the important studies of Dobriner and Lieberman (2) in which 90 mg. of testosterone per day were administered to a man for 45 days, metabolites equivalent to 50 per cent of this steroid were recovered from the urine.

Failure to find all of the injected testosterone as recognizable metabolites might have one of two explanations, or might include a combination of both. Either the steroid is degraded to non-steroidal, hence not easily recognizable, metabolites, or else only a portion of the metabolites of the injected material is eliminated via the urine.

That other pathways of excretion are important was demonstrated by the experiments of Paschkis et al. (3) in which significant amounts of androgenic material were found in the bile of dogs after injection of testosterone, androsterone, or methyltestosterone. However, Dorfman (1) reported that oral administration of large quantities of testosterone propionate to a woman with a bile fistula did not result in excretion of androgens or 17-ketosteroids in bile.

Since the initiation of the work reported in this paper, Gallagher et al. (4) have reported some studies on the metabolism of radioactive testosterone in rats and mice. After intraperitoneal administration of testosterone-4-C\textsuperscript{14} dissolved in aqueous propylene glycol no C\textsuperscript{14}O\textsubscript{2} was found in the expired air of mice during the succeeding 3 days. The distribution of radioactivity in the excreta of rats 24 hours after injection showed 7 to 12 per cent of the administered radioactivity in the urine, 31 to 40 per cent in the

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metabolites of testosterone are present in the gastrointestinal tract. After hydrolysis with boiling acid, only a minor proportion of the radioactive metabolites of the urine and feces was extractable with ether.

The experiments reported in this paper describe the preparation of testosterone-4-Cl\(^4\) and its metabolism by the rat. The labeled steroid was made by a modification of the method of Turner (5). Distribution of radioactivity in the excreta of normal male rats was measured following intragastric, intramuscular, or subcutaneous administration. Male rats having ligated or cannulated bile ducts were also used to study the rate of excretion of radioactive metabolites. Simple chemical studies were performed to determine the nature of the Cl\(^4\)-containing metabolites present in the bile and feces.

**EXPERIMENTAL**

**Preparation of Testosterone-4-C\(^4\)**—Testosterone-4-C\(^4\) was prepared by the method of Turner (5) with only slight variation. Ozonization of testosterone benzoate in which the 4,5 double bond is oxidized to give a ketone and carboxylic acid gave rise to a keto acid (m.p. 177–178\(^\circ\)) melting some 30\(^\circ\) higher than the compound reported by Turner. This higher melting acid was characterized by its neutral equivalent, specific rotation, and analysis for carbon and hydrogen. Table I gives a comparison of the properties found by us and those reported by Turner.

Hydrolysis of the benzoate of the keto acid produced an acid identical with the product derived from ozonization of testosterone. The melting point of the free acid, 202–203\(^\circ\), was depressed by admixture with both the keto acid benzoate and the enol lactone benzoate. These data, together with the fact that this acid gave an enol lactone identical with that reported by Turner, suggest that the higher melting keto acid benzoate is a dimorphic form.

The enol lactone was readily prepared by dissolving the keto acid benzoate in acetic anhydride, adding solid P\(_2\)O\(_5\), and refluxing the solution for 1 hour. After cooling, the solution was decanted from the dark red precipitate and evaporated to dryness. The residue was then dissolved in ether and the ether washed thoroughly with 7 per cent aqueous potassium carbonate and water. Evaporation of the ether yielded colorless crystals of the enol lactone, which upon crystallization from acetone-hexane gave a pure product melting at 201–202\(^\circ\); Turner reported 202–202.5\(^\circ\).

An excess of the enol lactone was condensed, as described by Turner, with 1.1 mm of phenyl acetate\(^1\) containing 1.1 mc. of Cl\(^4\). After adsorption on an alumina column and elution with benzene containing 1 per cent

\(^1\) The Cl\(^4\) was obtained on allocation from the Atomic Energy Commission and was purchased as methyl-labeled phenyl acetate from Tracerlab, Inc., Boston.
methanol, 0.58 mM of testosterone-4-C\textsuperscript{14} was obtained, representing a yield of 53 per cent. The radiotestosterone was characterized by its melting point (154–155\degree C) which showed no depression on admixture with authentic testosterone and by ultraviolet absorption, maximum 241 m\mu, log \epsilon 4.21. Testosterone benzoate was prepared as a derivative, m.p. 194–195\degree C; reported value, 194–196\degree C (6).

Radioassay of testosterone-4-C\textsuperscript{14} was carried out by oxidation of the material to carbon dioxide which was precipitated as BaCO\textsubscript{3} and counted with a thin window Geiger tube (1.8 mg. per sq. cm.). Comparison with a sample of C\textsuperscript{14} calibrated by the National Bureau of Standards revealed an activity of 2.1 \mu c. per mg.

**Excretion of Radioactivity in Bile, Urine, and Feces**—In all experiments, adult male albino rats from our colony, weighing in excess of 250 gm., were used. On the basis of the work of Moore and Price (7), 0.2 mg. (76,000 c.p.m.) was selected as being in the physiological range for the rat. After intragastric administration of the steroid in 1 ml. of 50 per cent ethanol or intramuscular injection in 0.2 ml. of dibutyl succinate, the excreta were collected for 4 days and the distribution of the radioactivity determined. Excretion in animals with cannulated or doubly ligated and severed bile ducts was studied only after intragastric administration. Operations were performed under ether anesthesia without rigid aseptic technique. Fistulas were made with metal-tipped polyethylene tubing, and these animals maintained in restraining cages (8).

The percentages of the injected doses excreted in bile, urine, and feces over a 4 day period are given in Table II. It is apparent from the total recoveries that from 86 to 104 per cent of the C\textsuperscript{14} of injected testosterone was accounted for in the excreta of the rats used in these experiments. Normal animals receiving 0.2 mg. of radioactive testosterone by either the intragastric or intramuscular route excreted from 44 to 72 per cent of the administered radioactivity in the feces and from 23 to 44 per cent in the urine.
Fig. 1 presents the daily excretion patterns following intragastric and intramuscular injection. By intragastric administration, normal rats (C) excreted more than 50 per cent of the injected radioactivity during the 1st day, with practically complete elimination by the end of the 2nd day. After intramuscular administration, the maximum excretion occurred on the 2nd day. Since 6 per cent was eliminated on the 4th day, it seems likely that absorption from the intramuscular site is much slower than from the intestinal tract.

<table>
<thead>
<tr>
<th>Type of rat</th>
<th>Recovery of injected C(^{14}) in excreta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
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<tr>
<td></td>
<td>per cent</td>
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<tr>
<td>Normal (IM)*</td>
<td></td>
</tr>
<tr>
<td>C-1</td>
<td>23</td>
</tr>
<tr>
<td>C-2</td>
<td>26</td>
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<td>C-3</td>
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<tr>
<td>Average</td>
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<tr>
<td>Normal (IG)†</td>
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<td>NC-7</td>
<td>29</td>
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<td>NC-8</td>
<td>44</td>
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<tr>
<td>NC-9</td>
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<tr>
<td>Average</td>
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<tr>
<td>Ligated bile duct (IG)</td>
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<td>DL-6</td>
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<td>DL-7</td>
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<td>Average</td>
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<td>Bile fistula (IG)</td>
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<td>BF-1</td>
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<td>BF-7</td>
<td>19</td>
</tr>
<tr>
<td>Average</td>
<td>16</td>
</tr>
</tbody>
</table>

* Administered intramuscularly in 0.2 ml. of dibutyl succinate.
† Administered intragastrically in 1 ml. of 50 per cent ethanol.
In animals with ligated bile ducts (DL), virtually all of the administered radioactivity appeared in the urine. The small percentage of activity in the feces of animals with cannulated or ligated bile ducts indicates practically complete absorption of testosterone from the intestinal tract, even when the flow of bile to the intestine has been interrupted, a finding which extends to the rat the previous report of Hoffman et al. (9). In animals with cannulated bile ducts (BF), the bile serves as the major route of excretion (72 to 88 per cent). The small percentage of radioactivity in the urine of animals with bile fistulas (5 to 28 per cent) as compared with that found in the urine of normal animals (23 to 44 per cent) indicates the resorption from the intestinal tract of metabolites which have been excreted in the bile.

Occurrence of Radioactivity in Expired Air—The expired air was collected from each of three normal animals which had received 0.2 mg. of radioactive testosterone intragastrically. Examination for C\textsuperscript{14}O\textsubscript{2} in the manner...
previously reported for progesterone by Grady et al. (10) revealed no radioactivity during the 4 day experimental period. In like manner, two additional male rats receiving 10 mg. (76,000 c.p.m.) and 40 mg. (76,000 c.p.m.) of radioactive testosterone subcutaneously showed no evidence of radioactivity in the expired air.

**Excretion of C\textsuperscript{14} after Subcutaneous Administration of 2 Mg. of Testosterone**

—2 mg. of the hormone in 0.2 ml. of dibutyl succinate were administered subcutaneously to two adult rats with cannulated bile ducts. Excretion of radioactivity in the bile, feces, and urine was followed for a 4 day period. The results of this experiment show the same over-all excretion pattern as was found on administration of 0.2 mg. of C\textsuperscript{14}-testosterone intragastrically. All of the administered radioactivity was recovered in the excreta within 3 days; 87 and 110 per cent in the bile and 16 and 2 per cent in the urine, respectively. No radioactivity was found in the feces.

**Enterohepatic Circulation in Rat**—Bile collected from animals receiving testosterone-4-C\textsuperscript{14} intragastrically was concentrated in the frozen state in \textit{vacuo} to one-fourth its original volume and administered intragastrically, intraduodenally, or intraejunally to other rats with cannulated bile ducts. The percentages of the injected doses recovered in bile, urine, and feces are given in Table III. That radiometabolites of administered bile were absorbed is evidenced by the reexcretion of from 42 to 65 per cent of the activity in the bile, with only from 5 to 6 per cent in the urine of these animals.

**Nature of Metabolites Containing C\textsuperscript{14} Excreted in Bile and Feces**—Simple chemical studies were performed to determine whether differences exist in the chemical properties of radioactive metabolites present in the excreta. The feces from animals receiving 0.2 mg. of testosterone intramuscularly were air-dried, pulverized, and continuously extracted with hot 95 per cent alcohol. The alcoholic extract was diluted with an equal volume of water and centrifuged at \(-4^\circ\). Aliquots of the supernatant were reduced to dryness and subjected to the following methods of hydrolysis: (1) boiling
with 15 volumes per cent of HCl (sp. gr. 1.19) for 10 minutes; (2) continuous extraction with ether of a 7.2 N HCl solution for 24 hours; and (3) action of bacterial β-glucuronidase at 37° for 24 hours (11).

A sample of bile from an animal receiving 0.2 mg. of radioactive testosterone intragastrically was divided into aliquot parts and subjected to the same hydrolytic methods that were used for fecal extracts. Ether extraction of the aqueous bile solution served as a control and conversion to ether solubility was taken as evidence of hydrolysis.

In agreement with Gallagher et al. (4), no significant quantities of radioactive material could be removed by ether extraction of the aqueous extract of feces either before or after hydrolysis. However, most of the metabolites present in bile were converted to ether-soluble forms after all hydrolytic methods. The hydrolysis of radioactive substances present in the bile by bacterial glucuronidase indicates that these substances are in part conjugated with glucuronic acid.

DISCUSSION

The data in Table II, Fig. 1, and the text show the same general pattern of excretion in rats, regardless of the route of administration or amount of hormone given. After either intragastric or intramuscular administration of radiotestosterone to normal rats, approximately two-thirds of the C\textsubscript{14} was excreted in the feces and one-third in the urine. The more rapid elimination after intragastric administration is indicative of a more rapid absorption from the gastrointestinal tract than from muscle.

In animals with cannulated bile ducts the bile contained an average of 79 per cent of the administered radioactivity, thus clearly establishing the hepatic route as the primary path of excretion in rats. Large percentages of activity found in the urine of animals with ligated bile ducts indicate that in impaired biliary excretion the kidney readily serves as an alternate pathway of excretion.

The percentage of radioactivity in the urine of normal animals (36 per cent) as compared with that of animals with cannulated bile ducts (16 per cent) indicates that the normal animal resorbs some of the C\textsubscript{14} from the gut after excretion into the bile. Resorption of testosterone metabolites from the intestinal tract is clearly demonstrated by the experiments in which bile containing radiometabolites was readministered. These experiments, which indicate significant enterohepatic circulation of metabolites of testosterone in the rat, support the concept proposed by Cantarow and associates (12-14), who studied steroid hormone excretion in the dog.

The absence of radioactivity in the expired air suggests that the isotope at position 4 remains with a large molecular residue and that a drastic breakdown of this steroid nucleus does not occur in the rat. The apparent difference in chemical behavior of radioactive metabolites present in the
bile and feces may be due to recycling through the animal organism (entero-hepatic circulation) or to alteration by intestinal flora.

**SUMMARY**

Testosterone-4-C¹⁴ has been synthesized by the method of Turner. In normal animals, approximately one-third of the administered radioactivity was excreted in the urine and two-thirds in the feces. In animals with cannulated bile ducts, it was found that the bile served as the major route of excretion, while in animals with ligated bile ducts, the kidney served as the major pathway of excretion. Significant amounts of the compounds excreted in the bile were resorbed from the intestinal tract when bile containing radiometabolites was administered to other bile fistula animals. No radioactivity was found in the expired air. Hydrolytic studies indicated that the radiometabolites in the feces were different from those in the bile.

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**BIBLIOGRAPHY**

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